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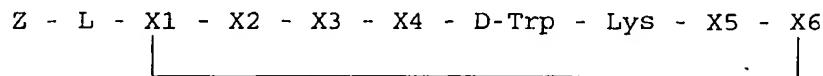
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(54) **Somatostatin analogues binding to all somatostatin receptor subtypes and their use**

(57) The present invention relates to novel somatostatin analogues of the general formula:



wherein Z may be absent or present and when present is selected from the group consisting of DOTA- and DTPA-based chelators, NOTA-based chelators, carbonyl compounds, hydrazino nicotinamide (hynic), N_xS_y -chelators, desferrioxamin, N_xS_y -chelators, all optionally complexed or labeled with a radioisotope, Tyrosine (Tyr) for halogenation, a fluorescent dye or biotin; L may or may not be present and is a linker molecule; X₁ is a symmetric or asymmetric diamino acid, containing 3 or 4 consecutive C atoms with a linker to the chelating agent, for example D/L-diamino butyric acid (D/L-Dab) for a more basic character or D/L-Glu for coupling to primary and secondary amino groups; X₂ is a positively charged natural or unnatural amino acid or arginine mimic or citrulline, or a neutral amino acid like Asn; X₃ is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α' - β -naphthylalanine; X₄ is an aromatic amino acid, optionally halogenated, in particular with Cl, Br, I or ¹⁸F.; X₅ is threonine (Thr) or serine (Ser); and X₆ is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α' - β -naphthylalanine.

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Description

[0001] The present invention relates to somatostatin analogues and their use in diagnosis and therapy. The invention also relates to pharmaceutical compositions comprising the novel analogues.

5 [0002] Somatostatin (somatotroph release-inhibiting factor), was initially discovered as a hypothalamic neurohormone that inhibits growth hormone secretion. It is a widely distributed peptide in both the central and peripheral nervous system and is also present in peripheral tissues including the endocrine pancreas, gut, thyroid, adrenals and kidneys. In addition, somatostatin is produced by inflammatory and immune cells as well as many cancer cells.

10 [0003] In mammals, two forms of bioactive peptides, somatostatin 14 and somatostatin 28 are found. They are produced by tissue-specific proteolytic processing of a common precursor. The natural somatostatin peptides have a short half-life, which is why many somatostatin analogues have been synthesized. Among them, octreotide, lanreotide and vapreotide have been intensively investigated and are in clinical use for the medical treatment of acromegaly and neuroendocrine tumors. These octapeptides retain the amino acid residues (or substitutes) within a cyclic peptide backbone that are involved in the biological effect of the peptide (Phe⁷ or Tyr⁷, D-Trp⁸, Lys⁹ and Thr¹⁰ or Val¹⁰) and display markedly increased stability.

15 [0004] The biological effects of somatostatin are mediated by specific plasma membrane receptors that have been identified in normal and neoplastic tissues by binding studies and receptor autoradiography techniques. Five somatostatin receptor genes have been cloned from human and mammalian libraries and designated sst1 to sst5 receptors. The sst subtypes belong to the family of G protein-coupled receptors with seven transmembrane-spanning domains and present a high degree of sequence identity (39-57%). The sequence differences reside in the extracellular and intracellular domains and are probably responsible for their signalling specificity.

20 [0005] All somatostatin receptors bind somatostatin 14 and somatostatin 28 with a high affinity (nM range), although with a slightly higher affinity for somatostatin 14. However, the receptors show major differences in their affinities for peptide analogues. Analogues that are known to date exhibit a low affinity for sst1 and sst4 whereas they bind the sst2 and sst5 receptor with a high affinity, comparable to that of somatostatin 14 and bind the sst3 receptor with moderate affinity.

25 [0006] In addition to its effect on secretion and intestinal motility, somatostatin inhibits the proliferation of normal as well as tumor cells. The antiproliferative action of somatostatin can be signalled via the five sst receptors which initiate pertussis toxin-sensitive G protein-dependent cell growth arrest or apoptosis according to receptor subtypes and target cells.

30 [0007] When expressed in CHO cells, ligand-activated sst1, sst2A, sst4, and sst5 receptors inhibit mitogenic signal of serum or growth factors as a result of hypophosphorylation of the retinoblastoma gene product (Rb) and G₁ cell cycle arrest.

35 [0008] However, distinct signal transduction pathways are involved in the somatostatin-induced G₁ cell cycle arrest depending on receptor subtype. The sst1 receptor mediates cell growth arrest through the stimulation of the tyrosine phosphatase SHP-2, activation of the Ras/MAP kinase ERK pathway and induction of the cyclin-dependent kinase inhibitor p21^{Waf1/Cip1}, whereas the sst5 receptor acts by a mechanism involving a dephosphorylation cascade leading to inhibition of guanylate cyclase, cGMP-dependent protein kinase G and MAP kinase ERK 1/2.

40 [0009] The antiproliferative effect mediated by the sst2 receptor results from the activation of the tyrosine phosphatase SHP-1 and the dephosphorylation of activated growth factor receptors thus leading to the negative regulation of growth factor-induced mitogenic signalling.

[0010] In addition, somatostatin-activated SHP-1 induces a G₁ cell cycle arrest, upregulates the cyclin-dependent kinase inhibitor p27^{Kip1} leading to the accumulation of hypophosphorylated Rb.

45 [0011] The antiproliferative effect of somatostatin can also result from apoptosis. Apoptosis is induced by sst3 as a result of the induction of p53 and Bax. In human pancreatic cancer cells expressing mutated p53 and devoid of endogenous sst2 receptor, correction of the deficit by expression of sst2 receptor induces an increase in cell death indicating that somatostatin can induce apoptosis by p53-dependent and p53-independent mechanisms.

50 [0012] The antiproliferative effects of somatostatin result from its actions via the endocrine pathway, but evidence exists that somatostatin can also act via an autocrine/paracrine pathway. Immunoreactive somatostatin has been found in somatostatin receptor-positive normal and tumor cell types such as endocrine, lymphoid cells, macrophages, breast cancer cells, colonic tumor cell and additionally, somatostatin mRNA is detected in a wide variety of neuroendocrine tumors known to express somatostatin receptors. Correction of the sst2 receptor deficit in human pancreatic cancer cells by sst2 receptor expression induces a negative autocrine loop in the absence of exogenous ligand, which is due to sst2 receptor-induced expression and secretion of endogenous sst2 ligand (somatostatin 14 and somatostatin 28).

55 This results in inhibition of cancer cell proliferation and reversion of cell tumorigenicity in vitro and in vivo after xenografts in nude mice.

[0013] The somatostatin effect on tumor growth may be the result of indirect effects of the peptide resulting from the inhibition of secretion of growth-promoting hormones and growth factors which specifically regulate tumor growth. For

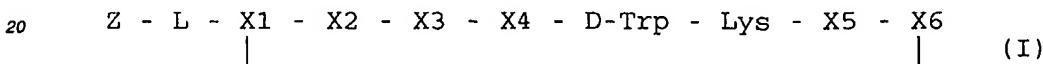
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example, the secretion of insulin-like growth factor-1 (IGF-1) which is produced by hepatocytes through GH-dependent and -independent mechanisms is negatively controlled by octreotide as a result of an effect on GH secretion and the sst₂ and sst₅ receptors have been demonstrated to be implicated in this effect. In addition, somatostatin can decrease IGF gene expression. Somatostatin also inhibits angiogenesis. Overexpression of peritumoral vascular somatostatin receptors with high affinity for somatostatin and octreotide has been reported in human peritumoral colorectal carcinomas, small cell lung carcinoma, breast cancer, renal cell carcinoma and malignant lymphoma. This expression appears to be independent of receptor expression in the tumor. It may reflect the presence of sst receptors in the venous smooth muscle cells as well as endothelial cells and may allow a vasoconstriction resulting in local hypoxia of the tumor or inhibition of endothelial cell growth and monocyte migration. Sst₂, sst₃ or sst₅ receptors might be involved in these effects.

[0014] Although the biological role and cellular distribution of each receptor subtype are not yet completely understood it is clear that the development of analogues binding to all somatostatin receptor subtypes, so-called pansomatostatin, has high potential.

[0015] It is thus the object of the present invention to provide such new somatostatin analogues that bind to all five somatostatin receptors and has a higher half-life (high metabolic stability) than somatostatin itself.

[0016] This is achieved according to the invention by somatostatin analogues of the general formula:



wherein:

25 Z may be absent or present and when present is selected from the group consisting of DOTA- and DTPA-based chelators, NOTA-based chelators, carbonyl compounds, 2hydrazino nicotinamide (hynic), N₄-chelators, desferrioxamin, N_xS_y-chelators, all optionally complexed with a radioisotope, Tyrosine (Tyr) for halogenation, a fluorescent dye or biotin;

30 L may or may not be present and is a linker molecule;
X₁ is a symmetric or asymmetric diamino acid, containing 3 or 4 consecutive C atoms with a linker to the chelating agent, for example D/L-diamino butyric acid (D/L-Dab) for a more basic character or D/L-Glu for coupling to primary and secondary amino groups;

35 X₂ is a positively charged natural or unnatural amino acid or arginine mimic or citrulline, or a neutral amino acid like Asn;

X₃ is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α-, β-naphthylalanine;

X₄ is an aromatic amino acid, optionally halogenated, in particular with Cl, Br, I or ¹⁸F;

X₅ is threonine (Thr) or serine (Ser); and

X₆ is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α-, β-naphthylalanine.

40 [0017] X₁ is preferably diamino propionic acid or diamino butyric acid.
[0018] Preferably X₂ is selected from the group consisting of Lysine (Lys), homolysine,

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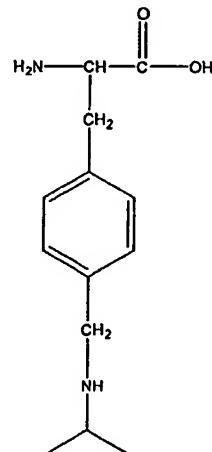
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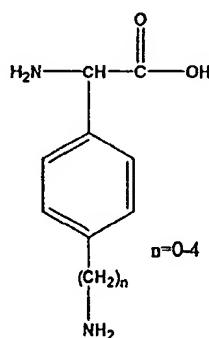
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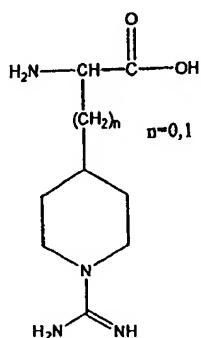


[0019] L/D-Phg(4-amidino) and derivatives. When X2 is an arginine mimic it is preferably a group selected from:

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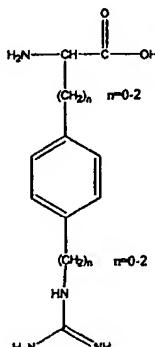
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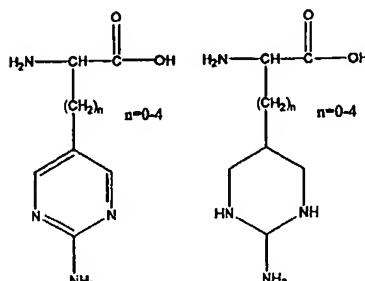


55 (R/S)-Gly/Ala-4-Pip(N-amidino)

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D/L-Phe[4-guanidino] and derivatives thereof



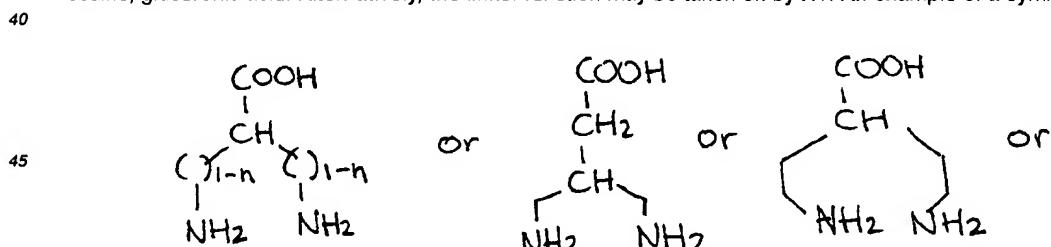
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(R/S)-2-amino-4-[4-(2-amino)-pyrimidiny]butanoic acid(n=2) and derivatives

and quarternary ammonium derivatives (NR_3^+).

[0020] X_4 is preferably selected from the group consisting of tyrosine (Tyr), halogenated tyrosine, in particular iodinated tyrosine (I-Tyr), dimethyltyrosine (diMe-Tyr), α - β -Naphthylalanine, halogenated phenylalanine, in particular iodinated phenylalanine (I-Phe). When X_4 is halogenated this is preferably with Cl, Br or I.

[0021] When a linker L is present, it may be selected from the group consisting of tyrosine, lysine, diaminobutyric acid, diaminopropionic acid, polyethylene glycol, fatty acids and their derivatives, β -alanine, 5-amino valeric acid, sarcosine, gluceronic acid. Alternatively, the linker function may be taken on by X_1 . An example of a symmetric linker is



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diamino acids, like diaminobutyric acid or 4,8 diaminoctanoic acid and symmetric molecules, like N,N-bis(N'-3-aminopropyl)glycine.

[0022] In case Z is complexed with a radioisotope, the isotope may be selected from the group consisting of ^{67}Ga , ^{68}Ga , ^{103}mRh , ^{195}mPt , ^{114}mIn , ^{186}Re , ^{188}Re , ^{77}As , ^{90}Y , ^{66}Ga , ^{67}Cu , ^{169}Er , ^{117}mSn , ^{121}Sn , ^{127}Te , ^{142}Pr , ^{143}Pr , ^{198}Au , ^{199}Au , ^{149}Tb , ^{161}Tb , ^{109}Pd , ^{165}Dy , ^{149}Pm , ^{151}Pm , ^{153}Sm , ^{157}Gd , ^{159}Gd , ^{166}Ho , ^{172}Tm , ^{169}Yb , ^{175}Yb , ^{177}Lu , ^{105}Rh , ^{111}Ag , ^{213}Bi , ^{123}I , ^{124}I , ^{125}I , ^{131}I and ^{211}At .

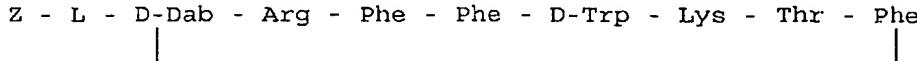
[0023] Preferred embodiments of the invention are somatostatin analogues of formula I wherein X_2 is arginine, or wherein X_3 is phenylalanine, or wherein X_4 is phenylalanine, or wherein X_4 is tyrosine, or wherein X_5 is threonine, or wherein X_6 is phenylalanine or wherein X_1 is diaminobutyric acid.

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[0024] The invention further relates to analogues wherein one or more of the above substituents are combined. In such analogues, the groups that do not have one of the above substituents are as defined for the general formula I above.

[0025] When all the above substituents are combined an analogue results having the general formula:

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wherein Z may or may not be present and is as defined above. In preferred embodiments of the invention Z is DOTA, DOTAGA or tyrosine.

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[0026] When X1 is D/L-Glu L are amine ending molecules like D/L-Lys, and Z are chelating agents like p-NH₂-Bz-DO-TA(2-p-aminobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), DOTA-p-NH₂-anilide (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(p-aminoanilide) and MeO-NH₂-Ph-DOTA (5-amino-2-methoxyphenyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid).

20

[0027] Z may be complexed with a radioisotope, or coupled to a fluorescent dye or biotin. For use in diagnosis the analogue may be labeled with a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ²⁰³Pb, ⁶⁷Ga, ⁶⁸Ga, ⁷²As, ¹¹¹In, ^{113m}In, ¹²³I, ¹⁷⁷Lu, ⁹⁷Ru, ⁶²Cu, ⁶⁴Cu, ⁵²Fe, ^{52m}Mn, ⁵¹Cr, ¹²⁴I and ¹⁸F.

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[0028] Suitable fluorescent dyes are cyanin-dyews. Such dyes may be used when the analogues are applied in *in vitro* and *in vivo* diagnosis. Biotin is useful as a label in histology.

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[0029] The somatostatin analogues of the invention can be used as a medicament in the treatment of diseases that are characterized by an overexpression of one or more somatostatin receptors, in particular the treatment may be directed to tumors bearing one or more somatostatin receptors. Examples of such tumours are neuroendocrinic tumors, astrocytoma, lung cancer, lymphoma, mama carcinoma, pancreatic tumors, thyroid cancer, colon cancer, SCLC, renal cell cancer.

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[0030] For therapy, the somatostatin analogues of the invention may be labeled with a radioisotope selected from the group consisting of ^{114m}In, ¹⁸⁶Re, ¹⁸⁸Re, ⁷⁷As, ⁹⁰Y, ⁶⁶Ga, ⁶⁷Cu, ¹⁶⁹Er, ^{117m}Sn, ¹²¹Sn, ¹²⁷Te, ¹⁴²Pr, ¹⁴³Pr, ^{195m}Pt, ¹⁹⁸Au, ¹⁹⁹Au, ¹⁴⁹Tb, ¹⁶¹Tb, ¹⁰⁹Pd, ¹⁶⁵Dy, ¹⁴⁹Pm, ¹⁵¹Pm, ¹⁵³Sm, ¹⁵⁷Gd, ¹⁵⁹Gd, ¹⁶⁶Ho, ¹⁷²Tm, ¹⁶⁹Yb, ¹⁷⁵Yb, ¹⁷Lu, ¹⁰⁵Rh, ^{103m}Rh, ¹¹¹Ag, ¹²⁴I, ¹³¹I and ²¹¹At.

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[0031] The invention also relates to the use of the somatostatin analogues for the preparation of a pharmaceutical composition for treatment or diagnosis.

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[0032] In case the pharmaceutical composition is a diagnostic composition, the somatostatin analogue(s) is(are) labeled with a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ²⁰³Pb, ⁶⁷Ga, ⁶⁸Ga, ⁷²As, ¹¹¹In, ^{113m}In, ¹²³I, ¹⁷⁷Lu, ⁹⁷Ru, ⁶²Cu, ⁶⁴Cu, ⁵²Fe, ^{52m}Mn and ⁵¹Cr. When the pharmaceutical composition is a therapeutical composition, the somatostatin analogue(s) is(are) labeled with a radioisotope selected from the group consisting of ^{114m}In, ¹⁸⁶Re, ¹⁸⁸Re, ⁷⁷As, ⁹⁰Y, ⁶⁶Ga, ⁶⁷Cu, ¹⁶⁹Er, ^{117m}Sn, ¹²¹Sn, ¹²⁷Te, ¹⁴²Pr, ¹⁴³Pr, ¹⁹⁸Au, ¹⁹⁹Au, ¹⁴⁹Tb, ¹⁶¹Tb, ¹⁰⁹Pd, ¹⁶⁵Dy, ¹⁴⁹Pm, ¹⁵¹Pm, ¹⁵³Sm, ¹⁵⁷Gd, ¹⁵⁹Gd, ¹⁶⁶Ho, ¹⁷²Tm, ¹⁶⁹Yb, ¹⁷⁵Yb, ¹⁷⁷Lu, ¹⁰⁵Rh, ^{103m}Rh, ^{195m}Pt, ¹¹¹Ag, ¹²⁴I, ¹³¹I and ²¹¹At.

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[0033] The invention furthermore relates to a pharmaceutical composition comprising a suitable excipient and one or more of the somatostatin analogues.

[0034] The present invention will be further elucidated in the following examples that are given for illustration purposes only and are in no way intended to limit the invention.

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EXAMPLES

EXAMPLE 1

General method for the synthesis of Chelator-Somatostatin analogues of the invention

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[0035] The synthesis to the linear peptide intermediates is performed with well-established solid phase methods with Fmoc as transient amino protecting group, and TFA-labile side chain protection groups as Pbf for Arg, BOC for Trp and Lys, tBu for Thr. For a description of the method see "Fluorenylmethoxycarbonylpolyamide solid phase synthesis-A practical approach" by E. Atherton and R.C. Sheppard, Information Press Ltd., Oxford, England (1989). The Solid phase peptide synthesis is carried out on a semiautomatic peptide synthesiser.

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[0036] The diaminobutyric acid is used with Fmoc as γ -amino protection group Z as α -amino protecting group. Coupling reactions are done with *in-situ* prepared hydroxy-benzotriazole esters with DIC, but any other coupling reagent may be used, e.g. the well introduced isourea derivatives (HATU etc.). The first amino acid (Fmoc-Phe-OH) is esterified

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to a super acid-labile linker (trialkoxy-benzhydryl or chloro-trityl) on the resin. After synthesis, the peptides are routinely cleaved mildly from the resin, using 20% acetic acid in dichloromethane. Coevaporation with toluene is performed two times leaving intact side-chain protection groups and therefore allowing subsequently cyclisation via carboxamide formation, using 10 equivalents of dicyclohexylcarbodiimide/hydroxybenzotriazole in DMF at high dilution.

5 [0037] The crude product was extracted three times between ethyl acetate and a 5% aqueous oxalic solution and the organic layer was concentrated to dryness. The Z-protecting group is then selectively removed by catalytic hydrog-
10 enolysis, using Pd/C as catalyst in methanol, without significant affection of the indole system. The products were filtered and purified with a SepPak cartridge C₁₈ (Macherey-Nagel, Düren, Germany) using water and methanol as eluents. The resulting free amino acid group serves as an attaching point for a carboxylic functionalised chelator de-
15 rivative, which may include an appropriate spacer. After the coupling to a prochelator, the peptide conjugate is depro-
20 tected with a solution of trifluoroacetic acid/phenole/thioanisol/water 85:5:5:5 for 2-5 hours. The final product was pre-
25 cipitated in isopropylether/petrolether 1:1 and purified by C₁₈ reverse phase chromatography (Metrohm LC CaDi 22-14, column: Macherey-Nagel, Düren, Germany) with a purity > 98%.

15 EXAMPLE 2

Synthesis of γ -9-fluorenylmethyloxycarbonyl- α -benzyl-oxy carbonyl-D-diaminobuc acid (Z-Dab(Fmoc)-OH)

20 [0038] α -Benzylloxycarbonyl-D-diaminobutyric acid (Z-Dab-OH) is commercially available (Bachem, Bubendorf, Switzerland). This starting material was dissolved in acetone/water 1:1, sodium carbonate was added to a final pH of 9-10 and treated with 9-fluorenylmethyloxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu). The reaction mixture was stirred for 18 hours at RT, cooled to 5°C; ethylacetate was added and then acidified with 6 N HCl. The organic phase was washed with water four times, dried over sodium sulfate and concentrated. The product was recrystallised from ethylacetate/petrolether.

25 EXAMPLE 3

Chelator coupling

30 [0039] Three equivalents of prochelator (for example DOTAGA(tBu)₄ (1-(1-carboxy-3-carbotertbutoxypropyl)-4,7,10-(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclo-dodecane), DOTA(tBu)₃ (1,4,7-tris(carbotertbutoxymethyl)-10-car-
35 boxymethyl-1,4,7,10-tetraazacyclododecane), DTPA(tBu)₄(1,1,7,7-tetrakis(carbotertbutoxymethyl)-4-carboxy-1,4,7-
triazapentane) were incubated with 3 equivalents of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-
hexafluorophosphate in DMF (dimethylformamide) for 30 min. The Z deprotected diamino acids (see above) were treated with this solution for 4 h at RT.

35 [0040] After concentration the mixture was dissolved in ethylacetate and extracted three times between 5% sodium hydrogencarbonate solution and ethylacetate. The organic layer was evaporated to dryness and the crude product is deprotected (see above).

40 [0041] A typical labeling procedure with ¹¹¹In and ⁹⁰Y is as follows. "PanSomatostatin" is one of the somatostatin analogues of the invention.

[0042] A buffer solution is prepared by dissolving 328 mg sodium acetate and 370 mg gentisic acid in 10 ml of water suprapure and adjusting the pH to pH 5 with 1 M sodium hydroxide.

45 [0043] 10 μ g of peptide (DOTA-PanSomatostatin), 100 μ l of buffer and 120 μ l of Yttrium-90 chloride (2.9 GBq/ml 0.05M HCl) were incubated at 95°C for 40 min, cooled to room temperature and the labeling yield and radiochemical purity was determined by RP-HPLC using a Macherey-Nagel Nucleosil-C₁₈ column and a linear gradient from 5% acetonitrile/0.1% trifluoroacetic acid to 60% acetonitrile/0.1% trifluoroacetic acid in 30 min. The labeling yield is >98%.

50 [0044] 10 μ g of peptide (DOTA-PanSomatostatin), 100 μ l of buffer and 100 μ l of Indium-111 chloride (370 MBq/ml 0.05M HCl) were incubated at 95°C for 40 min, cooled to room temperature and the labeling yield and radiochemical purity was determined by RP-HPLC using a Macherey-Nagel Nucleosil-C₁₈ column and a linear gradient from 5% acetonitrile/0.1 % trifluoroacetic acid to 60% acetonitrile/0.1% trifluoroacetic acid in 30 min. The labeling yield is >98%. The stability is >87% after one week in 10000 times excess of DTPA and 76 % in freshly prepared serum after one week, checked by HPLC. The serum proteins were precipitated with methanol, centrifuged and the methanol phase was injected, using the same gradient as above.

55 [0045] 10 μ g of peptide (DOTAGA-PanSomatostatin), 100 μ l of buffer and 20 μ l of Yttrium-90 chloride (1.8 GBq/ml 0.05M HCl) were incubated at 95°C for 40 min, cooled to room temperature and the labeling yield and radiochemical purity was determined by RP-HPLC using a Macherey-Nagel Nucleosil-C₁₈ column and a linear gradient from 5% acetonitrile/0.1 % trifluoroacetic acid to 60% acetonitrile/0.1 % trifluoroacetic acid in 30 min. The labeling yield is >98%. The radiochemical purity is >97% after 72h. The stability is >87% after 72h and >74% after one week in 10000 times

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excess of DTPA. Heating to 95°C for 45 min did not impair the radiopeptide.

EXAMPLE 4

5 Analysis of affinity profiles of various analogues for somatostatin receptors sst1-sst5

[0046] Cell culture CHO-K1 cells stably expressing human sst1 and sst5 were kindly provided by Drs. T. Reisine and G. Singh (University of Pennsylvania, Philadelphia, PA) and CCL39 cells stably expressing human sst2, sst3, and sst4 by Dr. D. Hoyer (Novartis Pharma, Basel, Switzerland).

10 [0047] CHO-K1 cells were grown in Ham's F-12 medium and CCL39 cells in Dulbecco's modified Eagle's medium/Ham's F-12 (1:1) mix, supplemented with 10% foetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin, in humidified air containing 5% CO₂ at 37°C. Geneticin (G418-sulfate; Gibco, USA) was used where necessary to maintain selection pressure at a final concentration of 400 µg/ml for sst2 to sst4 - and 285 µg/ml for sst5 - expressing cells as described previously [Rens-Domiano S & Reisine T. Biochemical and functional properties of somatostatin receptors. J. Neurochem. 58:1987-1996 (1992); OCaroll A et al., Characterization of cloned human somatostatin receptor SSTR5. Molec. Pharmacol. 46:291-298 (1994); Siehler S et al., [¹²⁵]Tyr¹⁰-cortistatin₁₄ labels all five somatostatin receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 357:483-489 (1998)].

15 [0048] All culture reagents were supplied by Gibco BRL, Life Technologies, Grand Island, NY.

20 EXAMPLE 5

In situ hybridisation histochemistry

25 [0049] To control adequacy of the cell material, in situ hybridisation for human sst mRNAs was performed on CHO-K1 and CCL39 cells expressing the different sst receptor subtypes.

[0050] Cells were detached from culture flasks by washing with Puck's Saline A and brief incubation with trypsin (0.5 mg/ml)/EDTA (0.2 mg/ml), collected by centrifugation, and resuspended in phosphate-buffered saline at a final cell density of approximately 6 x 10⁴ cells/µl. 25 µl Aliquots of cell suspension were spotted onto microscopic slides, air dried, and stored at -20°C.

30 [0051] They were subsequently fixed with 4% formaldehyde, washed with phosphate-buffered saline, air dried, and stored at 4°C under dry conditions. Cell smears were then used for sst1, sst2, sst3, sst4, and sst5 mRNA detection by in situ hybridisation. The protocol followed was essentially that described in detail previously [Reubi JC et al., Expression and localization of somatostatin receptor SSTR1, SSTR2 and SSTR3 mRNAs in primary human tumors using in situ hybridization. Cancer Res. 54:3455-3459 (1994)].

35 [0052] Oligonucleotide probes complementary to the sst1, sst2, sst3 [Reubi 1994, supra], sst4 and sst5 [Thoss VS et al., Expression of five somatostatin receptor mRNAs in the human brain and pituitary. Naunyn-Schmiedeberg's Arch. Pharmacol. 354:411-419 (1996)] mRNAs were synthesised and purified on a 20% polyacrylamide - 8M urea sequencing gel (Microsynth, Balgach, Switzerland). They were labelled at the 3'-end by using [α^{32} P]dATP (>3000 Ci/mmol; NEN Life Science Products, Boston, MA) and terminal deoxynucleotidyl-transferase (Boehringer, Mannheim, Germany) to specific activities of 33.3-74 GBq/mmol. Control experiments were carried out with the probes used in the present study to determine the specificity of the hybridisation signal obtained, as described previously [Reubi 1994, supra].

40 [0053] These control in situ hybridisation studies confirmed that the five cell lines used for the study expressed the correct sst mRNA.

45 EXAMPLE 6

Receptor autoradiography

50 [0054] Cells were washed twice with and scraped into ice-cold 0.05 M Tris-HCl (pH 7.4), collected by centrifugation, and homogenised using a rotor/stator slash system (Polytron, Kinematica Inc., Littau, Switzerland) in the same buffer. After centrifugation at 120 g for 5 min at 4°C, the supernatant was collected and centrifuged again at 48'000 g for 30 min at 4°C. The resulting pellet was resuspended in ice-cold Tris buffer, transferred into a microfuge tube, and centrifuged at 20'000 g for 15 min at 4°C. After withdrawal of the supernatant, the membrane pellet was stored at -80°C.

55 [0055] Receptor autoradiography was performed on 20µm thick cryostat (Leitz 1720, Rockleigh, NJ) sections of the membrane pellets, mounted on microscope slides, and then stored at -20°C. For each of the tested compounds, complete displacement experiments with the universal somatostatin radioligand [¹²⁵I- [Leu⁸, D-Trp²², Tyr²⁵] - somatostatin 28 using increasing concentrations of the unlabelled peptide ranging from 0.1-1000 nM were performed. The unlabelled, universal somatostatin 28 was run in parallel using the same increasing concentrations, as control.

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[0056] IC₅₀ values were calculated after quantification of the data using a computer-assisted image processing system as described previously [Reubi JC et al., Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. *Cancer Res.* 50: 5969-5977 (1990)]. Tissue standards (Autoradiographic [¹²⁵I] microscales, Amersham) that contain known amounts of isotope, cross-calibrated to tissue-equivalent ligand concentrations were used for quantification [Reubi JC. *In vitro* identification of vasoactive intestinal peptide receptors in human tumors: Implications for tumor imaging. *J. Nucl. Med.* 36: 1846-1853 (1995)]. Advantages of the present method using receptor autoradiography with sectioned cell pellets compared to binding on cell homogenates are, in addition to an economy on cells and a great flexibility, the greater inter-assay reliability and reproducibility, since the same embedded pellet can be used for successive experiments. As a minor disadvantage, IC₅₀ values are somewhat higher than in the homogenate binding assay.

[0057] The results are given in the following table. The compound tested is A:

15 Z - D-Dab - Arg - Phe - Phe - D-Trp - Lys - Thr - Phe
 [REDACTED]

wherein Z is varied.

20

| | Z | hsst1 | hsst2 | hsst3 | hsst4 | hsst5 |
|---------------------------------|-----------------|-------|-------|-------|-------|-------|
| ss28 ¹⁾ | - | 2.9 | 1.8 | 4.6 | 3.0 | 2.3 |
| 25 ss28 | - | 5.2 | 1.4 | 2.7 | 3.9 | 2.4 |
| ss28 | - | 5.2 | 2.8 | 3.8 | 5.7 | 3.8 |
| 30 A | DOTA | 7.6 | 3.0 | 1.6 | 0.9 | 0.8 |
| A | Y-DOTA | 3.5 | 3.7 | 0.9 | 2.1 | 3.2 |
| 35 A | Y-DOTA | 2.4 | 4.6 | 1.6 | 1.1 | 1.9 |
| A | Y-DOTA | 4.6 | 13 | 3.0 | 2.0 | 1.0 |
| 40 A | Ga-DOTA | 6.0 | 5.2 | 3.0 | 4.1 | 1.0 |
| A | DOTAGA | 40 | 3.8 | 0.8 | 3.0 | 1.5 |
| 45 A | DOTAGA | 24 | 3.8 | 2.5 | 2.3 | 1.9 |
| A | Y-DOTAGA | 64 | 7.5 | 1.0 | 8.0 | 3.1 |
| 50 A | Y-DOTAGA | 34 | 5.0 | 2.6 | 4.4 | 2.7 |
| A | Tyr | 1.8 | 0.6 | 1.4 | 1.3 | 0.48 |
| A with X4 is Tyr | NH ₂ | 24.5 | 2.9 | 2.0 | 1.0 | 0.8 |
| A with X4 is Tyr and X1 is GABA | - | 10.5 | 1.8 | 1.7 | 0.68 | 0.57 |

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| | | | | | | | |
|----|-------------------------------------|---|------|-----|-----|------|-----|
| 5 | minisoma tostatin ²⁾ | - | 172 | 2.8 | 3.2 | 15.3 | 6.8 |
| 10 | Arg- derivative ³⁾ | | 20.0 | 4.0 | 2.5 | 10.6 | 2.7 |

Control substances:

11) somatostatin 28

2) ab - Asn - Phe - Phe - D-Trp - Lys - Thr - Phe

15) ab - Arg - Phe - Phe - D-Trp - Lys - Thr - Phe

20) wherein ab is aminobutyric acid

[0058] It is preferred according to the invention that the IC₅₀ values range from 1 to 10 nM. From the above table it follows that the compounds of the invention show a IC₅₀ falling within the claimed range for most or all of the receptor subtypes.

25

Claims

1. Somatostatin analogues of the general formula:

30

Z - L - X1 - X2 - X3 - X4 - D-Trp - Lys - X5 - X6

35

wherein:

40) Z may be absent or present and when present is selected from the group consisting of DOTA- and DTPA-based chelators, NOTA-based chelators, carbonyl compounds, hydrazino nicotinamide (hynic), N₄-chelators, desferri-oxamin, N_xS_y-chelators, all optionally complexed or labeled with a radioisotope, Tyrosine (Tyr) for halogenation, a fluorescent dye or biotin;

45) L may or may not be present and is a linker molecule;

X1 is a symmetric or asymmetric diamino acid, containing 3 or 4 consecutive C atoms with a linker to the chelating agent, for example D/L-diamino butyric acid (D/L-Dab) for a more basic character or D/L-Glu for coupling to primary and secondary amino groups;

50) X2 is a positively charged natural or unnatural amino acid or arginine mimic or citrulline, or a neutral amino acid like Asn;

X3 is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α-,β-naphthylalanine;

X4 is an aromatic amino acid, optionally halogenated, in particular with Cl, Br, I or ¹⁸F;

X5 is threonine (Thr) or serine (Ser); and

X6 is phenylalanine (Phe), Ala-[3-(2-thienyl)] or α-,β-naphthylalanine.

55

2. Somatostatin analogues as claimed in claim 1, wherein X2 is selected from the group consisting of diamino propionic acid and diamino butyric acid.

3. Somatostatin analogues as claimed in claim 1 or 2, wherein X2 is selected from the group consisting of Lysine (Lys), I-Amp, ornithine and L/D-Phg(4-amino).

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4. Somatostatin analogues as claimed in claim 1 or 2, wherein X2 is selected from the group consisting of the arginine mimics (R/S)-Gly-Ala-4-Pip(N-amino), D/L-Phe[4-guanidino], (R/S)-2-amino-4-[4-(2-amino)pyrimi-dinyl]butanoic acid and quartemary ammonium derivatives (NR₃⁺).

5. Somatostatin analogues as claimed in claims 1-4, wherein X4 is selected from the group consisting of tyrosine (Tyr), halogenated tyrosine, in particular iodinated tyrosine (I-Tyr), dimethyltyrosine (diMe-Tyr), α -, β -Naphthylalanine, halogenated phenylalanine, in particular iodated phenylalanine (I-Phe).

6. Somatostatin analogues as claimed in claims 1-5, wherein L is selected from the group consisting of tyrosine, lysine, β -alanine, sarcosine, succinic acid, glutaric acid.

7. Somatostatin analogues as claimed in claims 1-6, wherein the radioisotope that is complexed with Z is selected from the group consisting of ^{114m}In, ¹⁸⁶Re, ¹⁸⁸Re, ⁷⁷As, ⁹⁰Y, ⁶⁶Ga, ⁶⁷Cu, ¹⁶⁹Er, ^{117m}Sn, ¹²¹Sn, ¹²⁷Te, ¹⁴²Pr, ¹⁴³Pr, ¹⁹⁸Au, ¹⁹⁹Au, ¹⁴⁹Tb, ¹⁶¹Tb, ¹⁰⁹Pd, ¹⁶⁵Dy, ¹⁴⁹Pm, ¹⁵¹Pm, ¹⁵³Sm, ¹⁵⁷Gd, ¹⁵⁹Gd, ¹⁶⁶Ho, ¹⁷²Tm, ¹⁶⁹Yb, ¹⁷⁵Yb, ¹⁷⁷Lu, ¹⁰⁵Rh, ¹¹¹Ag, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ²⁰³Bi and ^{103m}Rh.

8. Somatostatin analogues as claimed in claims 1-7, wherein X2 is arginine.

9. Somatostatin analogues as claimed in claims 1-8, wherein X3 is phenylalanine.

10. Somatostatin analogues as claimed in claims 1-9, wherein X4 is phenylalanine.

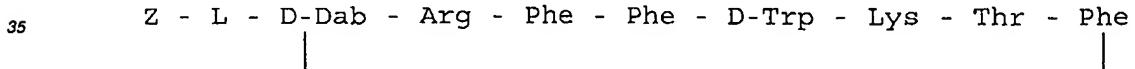
11. Somatostatin analogues as claimed in claims 1-9, wherein X4 is tyrosine.

12. Somatostatin analogues as claimed in claims 1-11, wherein X5 is threonine.

13. Somatostatin analogues as claimed in claims 1-12, wherein X6 is phenylalanine.

14. Somatostatin analogues as claimed in claims 1-13, wherein X1 is diaminobutyric acid.

15. Somatostatin analogues as claimed in claims 1-14 having the general formula:



40 wherein Z is as defined in claim 1.

16. Somatostatin analogues as claimed in claims 1-15, wherein Z is DOTA.

17. Somatostatin analogues as claimed in claims 1-15, wherein Z is DOTAGA.

18. Somatostatin analogues as claimed in claims 1-15, wherein Z is tyrosine.

19. Somatostatin analogues as claimed in claims 1-18, wherein L is absent.

20. Somatostatin analogues as claimed in claims 1-19 for use in therapy or diagnosis.

21. Somatostatin analogues as claimed in claims 1-19 for use in diagnosis and labeled with a radioactive (metal) isotope selected from the group consisting of ^{99m}Tc, ²⁰³Pb, ⁶⁷Ga, ⁶⁸Ga, ⁷²As, ¹¹¹In, ^{113m}In, ¹²³I, ¹⁷⁷Lu, ⁹⁷Ru, ⁶²Cu, ⁶⁴Cu, ⁵²Fe, ^{52m}Mn, ⁵¹Cr, ¹²⁴I and ¹⁸F.

22. Somatostatin analogues as claimed in claims 1-19 for use as a medicament in the treatment of diseases that are characterized by an overexpression of one or more somatostatin receptors.

23. Somatostatin analogues as claimed in claim 22, wherein the treatment is directed to tumors bearing one or more

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somatostatin receptors.

24. Somatostatin analogues as claimed in claim 22 or 23, labeled with a radioisotope selected from the group consisting of ^{114m}In , ^{186}Re , ^{188}Re , ^{77}As , ^{90}Y , ^{66}Ga , ^{67}Cu , ^{169}Er , ^{117m}Sn , ^{121}Sn , ^{127}Te , ^{142}Pr , ^{143}Pr , ^{198}Au , ^{199}Au , ^{149}Tb , ^{161}Tb , ^{109}Pd , ^{165}Dy , ^{149}Pm , ^{151}Pm , ^{153}Sm , ^{157}Gd , ^{159}Gd , ^{166}Ho , ^{172}Tm , ^{169}Yb , ^{175}Yb , ^{177}Lu , ^{105}Rh , ^{103m}Rh , ^{11}Ag , ^{124}I , ^{131}I and ^{211}At .

5 25. Use of somatostatin analogues as claimed in claims 1-19 for the preparation of a pharmaceutical composition for treatment or diagnosis.

10 26. Use as claimed in claim 25 wherein the pharmaceutical composition is a diagnostic composition and the somatostatin analogue(s) is(are) labeled with a radioactive (metal) isotope selected from the group consisting of ^{99m}Tc , ^{203}Pb , ^{67}Ga , ^{68}Ga , ^{72}As , ^{111}In , ^{113m}In , ^{123}I , ^{177}Lu , ^{97}Ru , ^{62}Cu , ^{64}Cu , ^{52}Fe , ^{52m}Mn and ^{51}Cr .

15 27. Use as claimed in claim 25 wherein the treatment is of diseases that are **characterized by** an overexpression of one or more somatostatin receptors.

28. Use as claimed in claim 27, wherein the treatment is directed to tumors bearing one or more somatostatin receptors.

20 29. Use as claimed in claims 27 and 28 wherein the pharmaceutical composition is a therapeutical composition and the somatostatin analogue(s) is(are) labeled with a radioisotope selected from the group consisting of ^{114m}In , ^{186}Re , ^{188}Re , ^{77}As , ^{90}Y , ^{66}Ga , ^{67}Cu , ^{169}Er , ^{117m}Sn , ^{121}Sn , ^{127}Te , ^{142}Pr , ^{143}Pr , ^{198}Au , ^{199}Au , ^{149}Tb , ^{161}Tb , ^{109}Pd , ^{165}Dy , ^{145}Pm , ^{151}Pm , ^{153}Sm , ^{157}Gd , ^{159}Gd , ^{166}Ho , ^{172}Tm , ^{169}Yb , ^{175}Yb , ^{177}Lu , ^{105}Rh , ^{111}Ag , ^{124}I and ^{131}I .

25 30. Pharmaceutical composition comprising a suitable excipient and one or more somatostatin analogues as claimed in claims 1-19.

31. Pharmaceutical composition as claimed in claim 30, wherein the composition is for use in diagnosis and the somatostatin analogue(s) is(are) labeled with a radioactive metal isotope selected from the group consisting of ^{99m}Tc , ^{203}Pb , ^{67}Ga , ^{68}Ga , ^{72}As , ^{111}In , ^{113m}In , ^{123}I , ^{177}Lu , ^{97}Ru , ^{62}Cu , ^{64}Cu , ^{52}Fe , ^{52m}Mn and ^{51}Cr .

35 32. Pharmaceutical composition as claimed in claim 30 wherein the composition is for treatment of diseases that are **characterized by** an overexpression of one or more somatostatin receptors.

33. Pharmaceutical composition as claimed in claim 32, wherein the treatment is directed to tumors bearing one or more somatostatin receptors.

40 34. Pharmaceutical composition as claimed in claims 32 and 33 wherein the pharmaceutical composition is a therapeutical composition and the somatostatin analogue(s) is(are) labeled with a radioisotope selected from the group consisting of ^{114m}In , ^{186}Re , ^{188}Re , ^{77}As , ^{90}Y , ^{66}Ga , ^{67}Cu , ^{169}Er , ^{117m}Sn , ^{121}Sn , ^{127}Te , ^{142}Pr , ^{143}Pr , ^{198}Au , ^{199}Au , ^{149}Tb , ^{161}Tb , ^{109}Pd , ^{165}Dy , ^{149}Pm , ^{151}Pm , ^{153}Sm , ^{157}Gd , ^{159}Gd , ^{166}Ho , ^{172}Tm , ^{169}Yb , ^{175}Yb , ^{177}Lu , ^{105}Rh , ^{111}Ag , ^{124}I and ^{131}I .

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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 01 20 3033
shall be considered, for the purposes of subsequent
proceedings, as the European search report

| DOCUMENTS CONSIDERED TO BE RELEVANT | | | CLASSIFICATION OF THE APPLICATION (Int.Cl.7) |
|--|--|---|--|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | |
| A | SCICINSKI J J ET AL: "The solid phase synthesis of a series of tri-substituted hydantoin ligands for the somatostatin SST5 receptor" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 8, no. 24, 15 December 1998 (1998-12-15), pages 3609-3614, XP004150376 ISSN: 0960-894X * the whole document * | 1-34 | C07K14/655 A61K38/31 |
| A | REUBI J C ET AL: "A SELECTIVE ANALOG FOR THE SOMATOSTATIN SST1-RECEPTOR SUBTYPE EXPRESSED BY HUMAN TUMORS" EUROPEAN JOURNAL OF PHARMACOLOGY, AMSTERDAM, NL, vol. 345, no. 1, 12 March 1998 (1998-03-12), pages 103-110, XP000876543 ISSN: 0014-2999 * the whole document * | 1-34 -/- | TECHNICAL FIELDS SEARCHED (Int.Cl.7) |
| | | | C07K A61K |
| INCOMPLETE SEARCH | | | |
| <p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p> | | | |
| Place of search: | Date of completion of the search | Examiner | |
| MUNICH | 21 January 2002 | G. Willière | |
| CATEGORY OF CITED DOCUMENTS | | | |
| <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> | | <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons B : member of the same patent family, corresponding document</p> | |
| ISO FORM 1523 (32 (P01/00)) | | | |

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European Patent
OfficeINCOMPLETE SEARCH
SHEET CApplication Number
EP 01 20 3033

Although claims 20-29 are directed to a diagnostic method practised on the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:

15

Claim(s) searched incompletely:

1-14, 16-19, 30-34

Reason for the limitation of the search:

Present claims 1-14, 16-19, 30-34 relate to an extremely large number of possible compounds/compositions. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is to be found, however, for only a very small proportion of the compounds/compositions claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

In addition the scope of claims 1-13, 16-19, 24-34 lacks clarity with regard to the definition of X1 representing on one side a diamino acid, and on the other side D/L-Glu which is not a diamino acid (see claim 1).

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds/compositions binding to all somatostatin receptor subtypes wherein X1 is represented by a diamino acid (pan-somatostatins).

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Application Number

EP 01 20 3033

| DOCUMENTS CONSIDERED TO BE RELEVANT | | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.) |
|-------------------------------------|--|-------------------|---|
| Category | Citation of document with indication, where appropriate, of relevant passages | | |
| A | <p>HOYER D ET AL: "Classification and nomenclature of somatostatin receptors" TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER TRENDS JOURNAL, CAMBRIDGE, GB, vol. 16, no. 3, March 1995 (1995-03), pages 86-88, XP004207465 ISSN: 0165-6147 * the whole document *</p> <p>-----</p> | 1-34 | |
| | | | TECHNICAL FIELDS SEARCHED (Int.Cl.) |